



Ozone effects on photosynthesis of ornamental species suitable for urban green spaces of China



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ABSTRACT

Urban green spaces (UGS) offer several ecosystemal benefits to the urban environment. However, these advantages may be weakened by alterations of plants photophysiological mechanisms due to increasing tropospheric ozone (O₃) concentrations, a serious problem for China. To evaluate their utilization in UGS, we selected three widely-used urban plant species (smoke tree, *Cotinus coggygria* Scop.; marigold, *Tagetes erecta* Linn.; rose, *Rosa chinensis* Jacq.) to investigate their biometric and photophysiological responses to (i) ambient air (AA), (ii) AA + 60 ppb O₃ (AA + 60), and (iii) AA + 120 ppb O₃ (AA + 120) (9 h d⁻¹, from 8:30 am to 5:30 pm). Considering visible injury and biomass production, smoke tree and marigold seem to be O₃-sensitive, whereas rose should be considered more tolerant. The exposure to the pollutant gas reduced photosynthetic efficiency in all seedlings. However, different features were shown throughout our study by the three species here monitored. In smoke tree, stomatal limitations seemed to be its principal weakness. In marigold, the reduction of the photosynthetic performance was mainly ascribable to impairments of both light and dark reactions of photosynthesis. Here, stomatal closure maybe not the cause to limit the photosynthetic rate, but a down-regulated response. Unexpectedly, CO₂ assimilation increased in roses exposed to AA + 60 and did not change in those treated with AA + 120 after one month from the beginning of the exposure (FBE). This seemed due to a better efficacy of these plants in dark reactions of photosynthesis. This feature was confirmed also a month later. In conclusion, the results of this study indicate that non-invasive methods such as gas exchange and chlorophyll fluorescence for monitoring photosynthetic performance of urban plants can be useful to give guidelines to manage UGS in the “climate change era”. Generally, in severe O₃-polluted areas as those of several cities of China, the plants with high-efficient biochemical processes driving a well photosynthetic performance (such as rose) might be a recommended choice.

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Abbreviations: A_{sat}, light-saturated photosynthesis; C_i, intercellular CO₂ concentration; %D, fraction of absorbed light thermally dissipated in PSII antennae; FBE, from the beginning of the exposure; F_v/F_m’, actual photochemical efficiency of PSII in the saturated light; g_s, stomatal conductance; J_{max}, light-saturated rate of electron transport; L_s, stomatal limitation to photosynthesis; M9, the daily 9-h mean O₃ concentration; NO_x, nitrogen oxides; OTC, open-top chamber; %P, fraction of absorbed light utilized in PSII photochemistry; Φ_{PSII}, light-adapted apparent quantum efficiency of PSII; PPF, photosynthetic photon flux density; PSII, photosystem II; qP, photochemical quenching; RH, relative humidity; RuBP, 1,5-diphosphate ribulose; TPU, triose phosphate utilization; UGS, urban green spaces; V_{max}, maximum rate of Rubisco-limited carboxylation; VOCs, volatile organic compounds; %X, fraction of light absorbed by *PSI* neither used in photochemistry nor dissipated in the PSII antenna.

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1. Introduction

Urban green spaces (UGS) offer several ecosystemal benefits to the urban environment, not only in relation to their aesthetic and social values but also for their effects on air quality (Pellegriani, 2014). Plants can beautify cities, improve eco-environment, promote living in harmony between human and nature, and contribute to public health, aesthetic enjoyment and physical and psychological well-being (Jo, 2002; Chen and Jim, 2008), suggesting their irreplaceable roles during urbanization. However, these advantages provided by UGS may be weakened by alterations of plants photophysiological mechanisms due to increasing tropospheric ozone (O₃) concentrations. Currently, O₃ has been proved to be one of the most toxic gaseous substances that significantly impacts plant life (Cotrozzi et al., 2016; Yi et al., 2016).

The fast economic development of China is producing serious air pollution problems. Large quantities of nitrogen oxides (NO_x) and volatile organic compounds (VOCs) are emitted from massive fossil fuel combustion of industrial activities and vehicular traffic. This phenomenon contributes to increasing ambient O_3 concentrations, especially in the regions with dense population, such as Beijing, Shanghai, and Guangzhou areas (Li et al., 2013; Zheng et al., 2014). Taking Beijing as an example, the daily mean (24-h) and hourly peak O_3 concentrations at urban and exurban regions were 46 and 67 ppb, and 181 and 209 ppb, respectively, during May–September 2010 (Feng et al., 2014) (for O_3 , 1 ppb = $1.96 \mu\text{g m}^{-3}$, at 25°C and 101.325 kPa). This confirms the well-known phenomenon of long range transport of O_3 precursors from urban to exurban areas, often leading to higher levels of the pollutant in regions far from cities or industrial zones (Lorenzini et al., 1995). Evenmore, the daily mean (24-h) O_3 concentration in May–September 2014 reached 71.3 ppb in exurban regions (Yuan et al., 2015). Thus, O_3 pollution has not been effectively controlled in recent years, and UGS are still living under high O_3 concentrations, expected to induce serious damages to plants.

Analysis and monitoring of plant responses to urban conditions, defined as “urban plant physiology” (Calfapietra et al., 2015) or “urban plant pathology” (Lorenzini and Nali, 2015), has been identified as a critical component of research programs. It represents an important opportunity to gain immediately an insight in relation to the physiological responses and the mechanisms (type and extent) of plant acclimation/tolerance. Current knowledge of plant photophysiology in the urban environment is mainly based on two non-destructive methodological approaches: (i) gas exchange analysis, and (ii) chlorophyll *a* fluorescence measurement.

Photosynthesis is the foundation of material cycle and energy conversion of plant, and is the primary carbon source for biomass formation. It is influenced by multiple step processes involving (i) carbon dioxide (CO_2) diffusion from atmosphere to leaf through stomata, (ii) light energy utilization and conversion (light reactions), and (iii) ribulose-1,5 biphosphate carboxylase/oxygenase (RuBisCo) carboxylation (dark reactions). Inhibition of any of these steps may affect the photosynthetic performance. Several experimental studies showed that O_3 stress induced decline in photosynthesis in parallel with decrease in stomatal conductance, while the intercellular CO_2 concentration remained constant or even increased, suggesting that stomatal closure may be only a downward-regulation response to decreased photosynthesis rather than the cause (Watanabe et al., 2014). Furthermore, several authors documented that the direct effect of O_3 on light and dark phases of photosynthesis was the main cause of its decline (e.g. Power and Ashmore, 2002). The detrimental effects of O_3 on potential photosystem II (PSII) photochemical efficiency (F_v/F_m), actual photochemical efficiency of PSII in the saturated light (F_v'/F_m'), light-adapted apparent quantum efficiency of PSII (Φ_{PSII}) and the percentage of open photosynthetic reaction centers (qP) have been widely reported (e.g. Feng et al., 2011b; Pellegrini, 2014; Zhang et al., 2014), suggesting that photochemistry was depressed and the production of NADPH and ATP for CO_2 reduction may be decreased. A reduction of maximum rate of RuBisCo-limited carboxylation (V_{cmax}) has also been considered a main factor being responsible for impairment of photosynthesis (Matyssek et al., 1991; Zheng et al., 2002; Morgan et al., 2004; Fiscus et al., 2005; Pellegrini et al., 2011). These are interesting findings to select and breed O_3 -tolerant species, but so far the photosynthetic mechanisms, including stomatal change, and light and dark reaction responses have not been explored enough.

Biomass production is to some extent the comprehensive reflection of photosynthetic capacity. Studies over the past several decades indicated that chronic O_3 exposure significantly decreased the production of forests (Proietti et al., 2016), crops (Avnery

et al., 2011; Ghude et al., 2014; Chuwah et al., 2015) and grasslands (Gilliland et al., 2016). However, there are some exceptions where O_3 exposure promoted biomass accumulation under particular environmental conditions (e.g. Prozhnerina et al., 2003). That photophysiological mechanisms lead to reverse biomass production responses to O_3 stress among different plant species has rarely been systematically explored.

Smoke tree (*Cotinus coggygria* Scop.), marigold (*Tagetes erecta* Linn.) and rose (*Rosa chinensis* Jacq.) have been widely used in urban landscaping (e.g. smoke tree contributed to 71% of Red Leaves, the most spectacular natural scenery in Fragrant Hills Park, an imperial garden in the northwestern part of Beijing), introduced in residential areas, parks, squares or on sides of roads. They are representative greening and ornamental plant species in cities. Before experiment, we consulted previous literatures to understand how these three species adapt to rising O_3 concentration through regulating photosynthetic mechanism. However, to the best of our knowledge, no study has been performed on this crucial topic. Only few reports on the photosynthetic efficiency responses of these species to other abiotic stresses were achieved (drought and high temperatures; van Iersel and Seymour, 2002; Li et al., 2011; Riaz et al., 2013) and, by the way, only the final photosynthetic rate was exhibited. Thus, we conducted this experiment to investigate the O_3 sensitivity (based on biomass production) of these three plant species, and to explore their crucial photophysiological mechanisms resulting in different biomass responses to elevated O_3 by analyzing stomatal factor along with photochemical and biochemical processes of photosynthesis in order to evaluate their utilization in UGS.

2. Materials and methods

2.1. Plant material and ozone exposure

Experiments were conducted at Zhangtuo village ($40^\circ 12' \text{N}$, $116^\circ 8' \text{E}$), Changping District, Beijing, China, where in 2013 and 2014 (the experimental period) the average annual temperature and precipitation were 12.1°C and 542 mm (concentrated from June to August), respectively (Chen et al., 2016).

Seedlings were purchased from a local farmer (smoke trees were one-year old, whereas rose and marigold were just emerged in the current year when relative experiments were conducted), and individually planted into plastic pots (17 cm in height and 22 cm in diameter) filled with native soil. About 10 days later, when plants were adapted to pots conditions, 30 seedlings of smoke tree and 60 of rose and marigold, each, with similar height (102, 24 and 7 cm for smoke tree, rose and marigold, respectively) and stem diameter (12.5, 8 and 4 mm, respectively) were selected and equally distributed to three octagonal open top chambers (OTCs; 2.8 m in height and 4.0 m in diameter), made of aluminum alloy frame covered with stalinite; (Zheng et al., 2011). Three treatments were applied (one for each OTC): (i) ambient air (AA), (ii) AA with the addition of 60 ppb O_3 (AA+60), and (iii) AA with the addition of 120 ppb O_3 (AA+120) (for O_3 , 1 ppb = $1.96 \mu\text{g m}^{-3}$, at 25°C and 101.325 kPa). O_3 was generated from pure oxygen by an O_3 generator (HY003, Chuangcheng Co., Jinan, China) using a high voltage discharge method (Zheng et al., 2013), and then mixed with AA to achieve the target concentration. Depending on the growth season of each species, smoke tree, rose and marigold seedlings were exposed to O_3 (9 h d^{-1} , from 8:30 am to 5:30 pm) from 23 June to 19 October 2013 (118 days), 23 June to 29 October 2013 (128 days), and 5 August to 28 September 2014 (54 days), respectively (with exception of rainy and windy days). During exposure period, the average temperature, relative humidity (RH) and precipitation were 24.3°C , 77.3% and 365.2 mm for smoke tree and rose, respectively, and

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